Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008;359:584-92.

WOLF 07-06130

Supplemental Data

Protection of Human Subjects

All blood samples that were collected from incident hemodialysis patients were sent to a central laboratory (Spectra Laboratories, NJ). After these samples were processed for their primary clinical use, the remaining blood samples that were to be discarded were retained, de-identified after processing by Spectra, and each was assigned a random number. No extra blood was drawn from any patient for the study, and all routine clinical tests for which the samples were collected were performed by the central laboratory before any sample was saved for the ArMORR study. A HIPAA Limited Data Set (LDS) was created by Fresenius, including only incident patient data, and stripped of any HIPAA identifiers. Fresenius assigned the same random number of the blood sample to patient data before any data or samples were transferred to the ArMORR investigators. Once all data and blood samples were collected during the one year of follow-up the study was terminated leaving a dataset of de-identified patients, but with random numbers linked to discarded blood samples that were stored in the ArMORR freezers. The Partners IRB did not consider ArMORR human subjects research because no identifiers were retained, HIPAA LDS directives were explicitly followed, and all biological samples were de-identified and discarded after their original clinical use. Hence, the need for written informed consent was waived. Although an external audit of the Partners IRB similarly deemed ArMORR exempt from human subject approval, we have maintained an active IRB approval since 2004 including annual continuing reviews.

Post-hoc analyses of additional controls

In order to ensure that the controls selected for this study were adequately representative of all possible controls that could have been selected under the risk-set sampling theory, we conducted additional analyses. First, we compared baseline demographic and laboratory data between the 200 controls who were selected for the study and the potential controls who were excluded from eligibility owing to transplant, recovery of kidney function, or transfer to a non-FMC unit prior to completing one year of follow-up (n = 1944; 19%).

Variable	Controls (N=200)	Censored (N=1944)	P
Age	61 ± 15	60 ± 16	0.24
Female (%)	46	43	0.49
Black (%)	36	28	0.02
Hispanic (%)	15	11	0.14
Body mass index (kg/m^2)	28 ± 6	28 ± 11	0.51
Blood pressure (mm Hg)			
Systolic	146 ± 21	145 ± 22	0.37
Diastolic	75 ± 14	76 ± 14	0.51
Etiology of renal failure (%)			
Diabetes	44	40	0.28
Hypertension	37	33	0.24
Glomerulonephritis	11	13	0.44
Other	8	12	0.21
Co-morbidities (%)			

Coronary artery disease	8	8	0.67
Congestive Heart Failure	10	10	0.9
Albumin (g/dl)	3.5 ± 0.5	3.4 ± 0.6	0.19
Calcium (mg/dl)	8.9 ± 0.7	8.9 ± 0.8	0.63
Phosphate (mg/dl)	4.4 ± 1.7	4.7 ± 1.6	0.01
PTH (pg/ml)	187 (97-323)	198 (113-343)	0.12

As shown in the Table, the populations were similar across a broad range of baseline variables, though a few differences were noted. Black patients constituted a greater percentage of patients who were selected for the study compared to individuals who were censored because we specifically excluded other race/ethnicities from the study (n=693). In addition, although phosphate levels were lower in the controls selected for the study than in patients that were censored, the overall difference between the groups was small. The similar characteristics of the groups suggest that the 200 controls selected for the study were representative of the overall population of possible controls that could have been selected under the risk-set sampling theory.

Next, we measured cFGF-23 and iFGF-23 levels in 50 new subjects from the ArMORR cohort in order to determine if FGF-23 levels differed between controls selected for the study and potential controls that were excluded. The 50 new controls were randomly selected from the population of censored controls to meet the following criteria: (1) the new controls were alive at the time cases died and frequency matched by the timing of their being censored to the timing of death in the cases so as to ensure that their follow-up time was similar to the cases but their outcomes different—i.e., since 40% of cases died within the first calendar quarter after initiating dialysis, 40% (n = 20) of the new controls who were alive but were censored after this quarter were

selected. Similarly, since 23% of cases died between 3 and 6 months after initiating dialysis, 24% (n = 12) of the new controls who were alive but censored after this period were selected, and so forth; and (2) the 50 new controls were equally distributed across the four quartiles of baseline serum phosphate (13 in quartile 1, 12 in quartile 2, 13 in quartile 3, and 12 in quartile 4).

Median cFGF-23 levels in the new controls were not statistically different from median cFGF-23 levels in the controls selected for the study (1684 [IQR 662, 3300] vs. 1406 [IQR 989, 2740] RU/ml, P = 0.34). Similarly, median iFGF-23 levels in the new controls were not statistically different than iFGF-23 levels in the original controls (729 [IQR 594, 1083] vs. 664 [IQR 536, 854], P = 0.93). Thus, the 50 new controls had similar FGF-23 levels compared to the original 200 controls selected for the study.

Finally, the association between cFGF-23 and iFGF-23 and mortality remained qualitatively unchanged when we repeated the logistic regression analyses including the 50 new controls in the models:

Odds ratio of mortality according to quartiles of c-terminal FGF-23:

	Original analysis	**Original Analysis + 50 new controls**
	OR (95% CI)	**OR (95% CI)**
Quartile 1	ref	ref
Quartile 2	1.5 (0.9, 2.7)	1.8 (1.0, 3.9)
Quartile 3	2.5 (1.4, 4.4)	2.5 (1.4, 4.2)
Quartile 4	3.4 (1.9, 6.9)	3.3 (1.9, 5.8)

Odds ratio of mortality according to quartiles of intact FGF-23:

	Original analysis	**Original Analysis + 50 new controls**
	OR (95% CI)	**OR (95% CI)**
Quartile 1	ref	ref
Quartile 2	1.8 (0.9, 3.2)	1.6 (0.9, 2.8)
Quartile 3	2.2 (1.2, 3.9)	2.0 (1.2, 3.4)
Quartile 4	3.3 (1.8, 5.8)	2.4 (1.4, 4.2)

In summary, baseline demographic and laboratory data were similar between the controls selected for the study and those who were excluded because of incomplete one-year follow-up. FGF-23 levels did not differ significantly between the 50 new controls and the 200 controls selected for the primary analysis, and when the 50 new controls were added to the logistic regression models, the odds ratio of mortality for each quartile of iFGF-23 and cFGF-23 remained qualitatively unchanged.

Supplemental Tables

Supplemental Table 1.

Laboratory results according to quartiles of intact FGF-23 levels (pg/ml).

1,25-dihydroxyvitamin D levels were available in 121 patients. Results are reported as mean ± standard deviation, median (interquartile range), or proportions, as appropriate. P values refer to tests for linear trend.

	iFGF-23 Quartile 1 < 575	**iFGF-23 Quartile 2 576 – 715**	**iFGF-23 Quartile 3 716 – 950**	**iFGF-23 Quartile 4 > 950**	**P**
Albumin (g/dl)	3.3 ± 0.6	3.3 ± 0.6	3.4 ± 0.5	3.3 ± 0.5	NS
Creatinine (mg/dl)	5.8 ± 2.2	5.7 ± 2.5	5.6 ± 2.2	6.5 ± 2.9	NS
Phosphate (mg/dl)	4.3 ± 1.3	3.9 ± 1.4	4.4 ± 1.6	5.2 ± 2.0	<0.01
Calcium (mg/dl)	8.9 ± 0.9	8.9 ± 0.6	8.7 ± 0.7	9.1 ± 0.8	NS
Bio-intact PTH (pg/ml)	205	159	186	233	NS
	111 – 335	89 – 299	102 – 335	134 – 389	
Alkaline phosphatase (U/L)	89	87	87	91	NS
	68 – 118	67 – 112	65 – 115	76 – 127	
1,25-dihydroxyvitamin D (pg/ml)	8.7 ± 5.5	7.6 ± 6.4	7.4 ± 4.9	8.3 ± 6.3	NS
Phosphorus binders (%)	12	9	9	6	NS

Supplemental Table 2.

Intact FGF-23 levels in patients who died as compared with those who survived

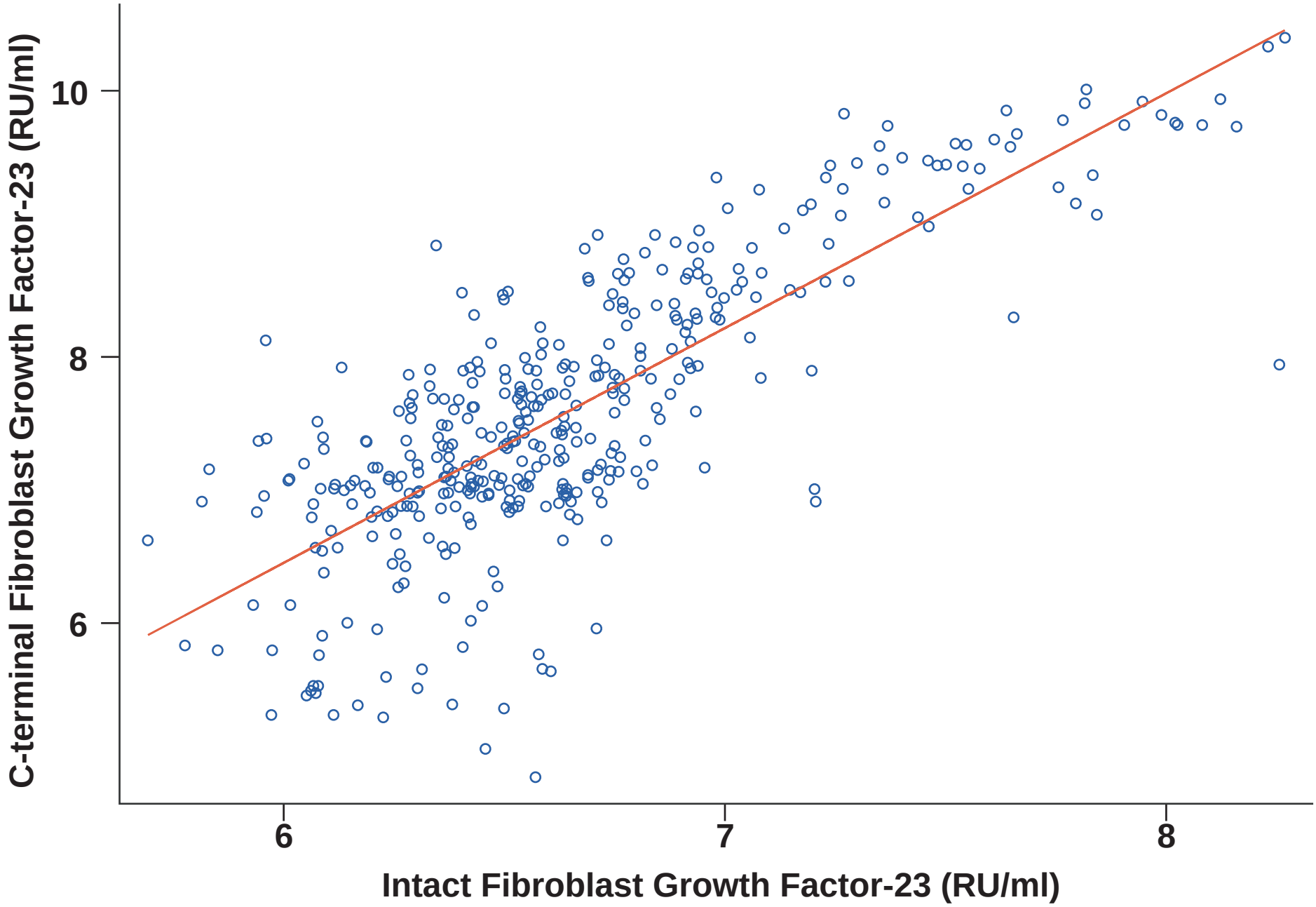
The odds ratio for mortality is expressed according to 1 unit increase in log-transformed iFGF-23 levels. In the left columns of the table, FGF-23 levels are reported as median, interquartile range in the overall case-control sample and within individual serum phosphate quartiles. In the right column of the table, odds ratio of morality and 95% confidence intervals are similarly reported. 50 cases are compared to 50 controls in each phosphate quartile.

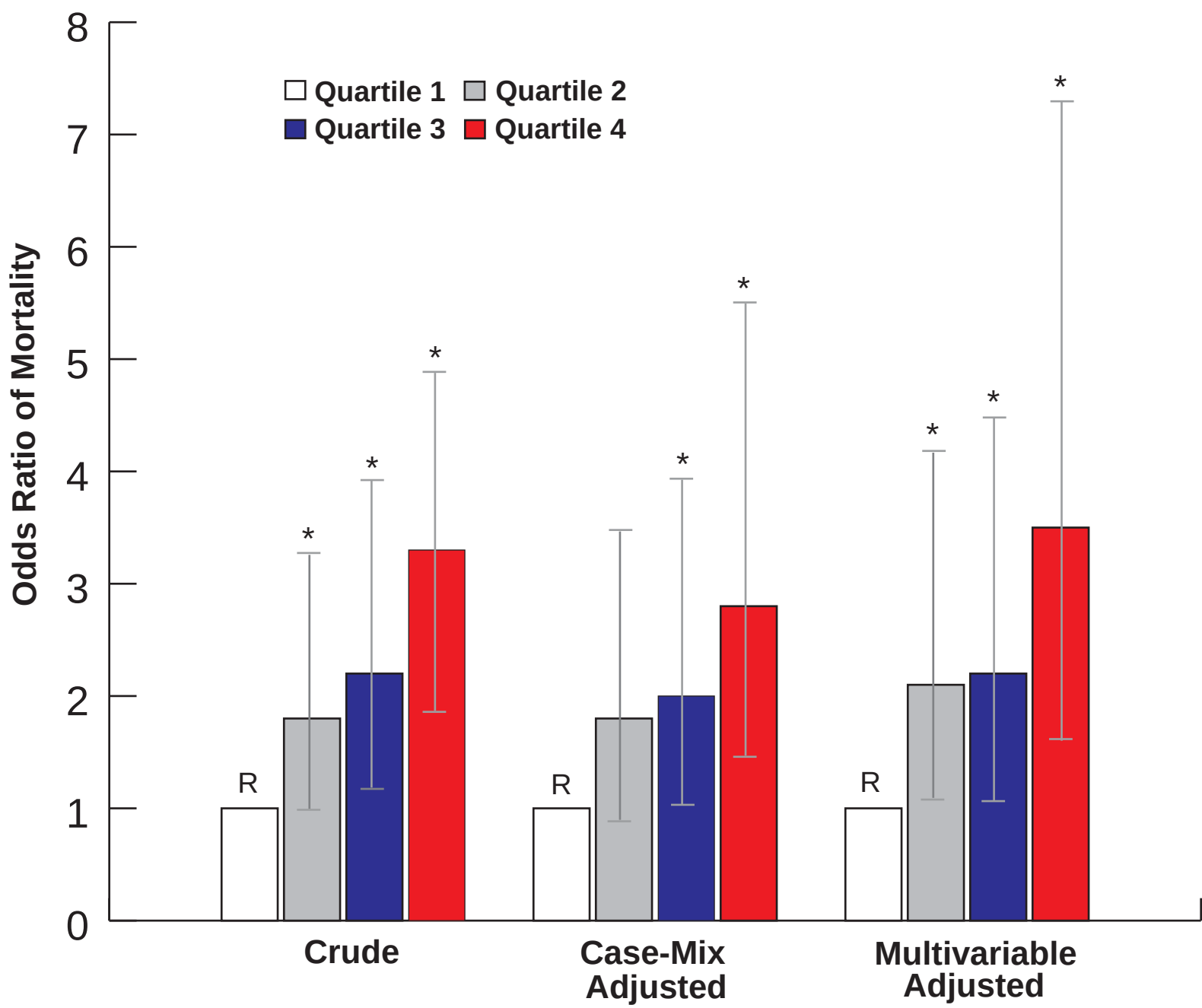
Phosphate (mg/dl)	**Died N=200**	**Survived N=200**	**P**	**Odds ratio of mortality/unit increase in log iFGF-23**
All patients	763	664	< 0.01	2.0
	618, 1032	536, 854		1.3, 3.1
< 3.5	690	655	0.07	3.4
	616, 893	570, 761		1.1, 10.3
3.5 – 4.4	757	610	< 0.01	4.1
	632, 1020	506, 766		1.5, 11.0
4.5 – 5.5	744	621	< 0.01	4.2
	586, 1056	537, 761		1.4, 13.2
> 5.5	864	862	0.61	0.9
	702, 1574	606, 1308		0.5, 1.8

Supplemental Figure Legends

Supplemental Figure 1. Correlation between C-terminal and intact FGF-23 levels ($r = 0.74$, $P < 0.001$).

Supplemental Figure 2. Crude, case-mix-adjusted, and multivariable-adjusted odds ratio of mortality according to quartiles of iFGF-23 (Quartile 1, < 575 pg/ml; Quartile 2, 575-715 pg/ml; Quartile 3, 716-950 pg/ml; Quartile 4, > 950 pg/ml). The case-mix-adjusted analysis included the following variables: age, sex, race, ethnicity, BP, BMI, SMR, vascular access, history of diabetes, and congestive heart failure. The multivariable-adjusted analysis included the case-mix variables plus phosphate, calcium, log PTH, albumin, creatinine, and ferritin. Quartile 1 is the reference group in all models. Vertical lines represent 95% confidence intervals.


C-terminal Fibroblast Growth Factor-23 (RU/ml)
10
8
6
6
7
8
Intact Fibroblast Growth Factor-23 (RU/ml)



Quartile 1	Reference	Reference	Reference
Quartile 2	1.8 (1.0, 3.2)	1.8 (0.9, 3.4)	2.1 (1.1, 4.1)
Quartile 3	2.2 (1.2, 3.9)	2.0 (1.1, 3.8)	2.2 (1.1, 4.3)
Quartile 4	3.3 (1.8, 5.8)	2.8 (1.5, 5.5)	3.5 (1.7, 7.2)